Steven M. Ruben
Appl. No. 10/662,429

BEST AVAILABLE COPY

Ruben EXHIBIT #70

Department Protein Expression

Subject

Name Flavio Benedike Jorina

Address

National Brand 43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

0 73333 43648 8

Ruben EXHIBIT 2070
Ruben v. Wiley et al.
Interference No. 105,077
RX 2070

REDACTED

Transfection — Sf9 cells — 1/27/94

Prepare dishes 1x 35 mm ø with 1 to 1.5 x 10^6 Sf9 cells/ml in Grace's media + 5% inactivated F.B.S. Incubate overnight 27°C on Sf 900

Ob: my cells were prepared with 10% H.I F.B.S

Check the plate to see if the cells are OK
Remove the media carefully

Add 1 ml of media without FBS 1 or 2 hrs

Prepare the transfection mix

A: 10 µl of Lipofectin
90 µl of Media without FBS

B: 50 µl of Media without FBS
5 µl of plasmid pc3 (A2) at 1 µg/µl
pc4 (3+)
5 µl of Linearized Baculogold DNA [0.1 µg/µl]

Mix the contents of tube A and B - and allow the mixture to stand at least 15 minutes at Room Temperature before adding it to the cells

Remove the culture dish from the incubator and add the transfection mixture to it drop wise around the plate

Shake the plate forward and sideways and incubate again 27°C for 4 to 6 hours

After this time add 1 ml of Media containing 10% of H.I FBS and switch and incubate for 3 to 4 days or until some polyhedrin are visible

1.30.94 Add 4 ul Xgal (stock solution 20mg/ml) each plate (dish)

- Harvest 1 ml of supernatant into a sterile epp

Spin at top speed to pellet any cells and debris

- Transfer the supernatant into labeled sterile epp tube and store at 4°C

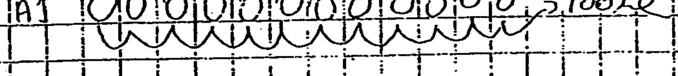
- first stock

Clone Purification

Prepare a flat bottom 96 well with 100 ul/well of Sf9 cells at 1.5 to 2×10^6/ml

in SF 900 media 1% serum H.I. or
Grace's media 10% [illegible] H.I.
add 40 ug/ml (200 ul in each 10 ml)
X-Gal

- Add 100 ul of the transfected viral supernatant to well A1 in the multi well plate



- Add 100 ul of media to well A1 to A12
- transfer 100 ul to well A to B, C etc with multichanel pipt
- Return the plates to incubate at 2 to c for about 4 to 5 days or until the blue color becomes visible across the plate
- make a photo copy of the plate, chose a well at the highest virus dilution which gives a strong blue staining reaction

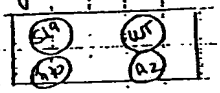
2 | 94 2 prepared again 2 microtiter plates 96 well

SF 900 media + 2% H.I Serum + X gal (200 ul / 10 ml) dilution according to the protocols

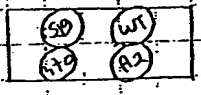
Virus → SC3 A12
SC4 mTO

2 | 94 6 well plates → prepared with H.I Serum for labelling

Grace's 10%



SF 900 2%



2 ml of media each well
50 ul of each virus/well

2 4 94 First labelling 3+ 5 cysteine
↳ Scott made it 3+ 5 Met

Procedure:

- Remove media (complete) from 6 well plates prepared 2 |

add 1.5 ml SF 900 II (- cys - met - serum)
without

incubate 1 hour

add 10 ul ^{35}S cysteine each well
10 ul ^{35}S met " "

after 2 hours remove

50 ul → add sample buffer → fuge
200 ul → stock
-20°C

after 5 hours do the same like above
after 24-28 hr remove the rest

- 50 ul → + sample buffer
- 800 ul → stock -20°C
+ 100 ul → Harvest cells

Protein Gel → Scott
Sample preparation

2/9/94 Prepared 6 wells for labelling → 2nd → 2/12/94 → Reimer did it

Sf 900 II + 2% H.I Serum

REDACTED → Sf900 II
→ Graces 10% H.I.S

cells seeded in 2 ml media + 40 ul of each virus from 1st stock

2/13/94 Collected samples:

1	wt	sup.	SF900	5	SC4	SF900		
2	"	"	Grace's	6	SC4	Graces		
3	SC3		SF900	7	cells	wt	SF900	
4	SC3		Graces	8	"	"	Graces	

9: cells SC3 SF900
10: " SC3 Graces
11 " SC4 SF900
12 " SC4 Graces

Reimer did it ←

2 9 94 Prepared 6well dish for infection

REDACTED

REDACTED

media SF 900 II + 2% FBS H.S. → 2ml

REDACTED

2 18 94 Collect supernatant → virus Stock

2 18 94 - Add new media to the plate above

2 18 94 Prepared 2 dishes 6well for

a) labeling: infected with virus STOCK collect 2 18 94

SF900II media REDACTED

b) another infection: in Grace's media with virus Stock from 2 18 94

REDACTED

2 23 94 → Collected the third stock of virus

REDACTED

2 23 94 → Infection big flask 0.5 ml of C5 stock virus from 2 23 94 (grace's 10% H.S.)

2 23 94 ⇒ third labelling → Neiner did it

2 24 94 → Samples labelled (2.23) were collected
- spin 30'
- collect SPN
- freeze -20°C

REDACTED

6

2.25.94 collected stock of virus CS infected 22394 in Grace's 10% H.I.S.

3.1.94 PLAQUE PURIFICATION OF BACULOVIRUS STOCK

CS stock from 2.25.94

E12 from Sf900 plate Nam 2.1.94

Protocol → from Gibco - BRL's methods

PLAQUE PRODUCTION

Materials:

In addition to the materials listed in *General Information*, page 5, you will need the following:

Exponentially growing Sf9 cells (viability >90%)

GIBCO BRL 4% Agarose Gel (or GIBCO BRL 4% Agarose Gel with Bluo-gal)

GIBCO BRL Sf-900 Medium, 1.3X or GIBCO BRL Grace's Insect Medium, 2X; HI-FBS; and sterile, deionized, distilled water

Sealable plastic container (~4" × 8" × 8")

Automated pro-pipette or other suction/reservoir system

60-mm cell culture dishes

27°C incubator

70°C water bath

37°C water bath

Protocol:

1. Prepare >1 ml of each of the log serial dilutions of the virus to be titered or purified from 1×10^{-2} through 1×10^{-6}.
2. Prepare a 50-ml stock suspension at 5×10^5 cells/ml using Sf9 cells from an exponentially growing culture at >95% viability.
3. Dispense 4 ml of this suspension into 60-mm cell culture dishes, swirling gently while dispensing to insure even dispersal of cells.
4. Allow cells to settle to bottom of plate for 1 h. Note: In serum-containing media, transport the plates gently because cells do not adhere tightly to the plate surface.
5. Inspect the cultures on an inverted microscope to confirm a 50% confluency. Adjust density as necessary.
6. Sequentially remove supernatant medium from each plate with a pipette and replace with 1 ml of a dilution of the virus. Incubate at room temperature for 1 h, initially tipping the plates back and forth every 5 min to insure even adsorption of the virus.
7. While plates are incubating, melt the 4% Agarose Gel completely in a 70°C water bath (~10 min) and maintain at 37°C.

To prepare the Grace's plaquing overlay:

8. Place a bottle of sterile, deionized, distilled water and an empty, sterile, 100-ml glass container in a 37°C water bath. Add, under sterile conditions, 20 ml of HI-FBS to a bottle of Grace's Insect Medium, 2X. Mix by pipetting, and move the mixture to 37°C water bath.
9. Combine 25 ml of Grace's Insect Medium, 2X; 12.5 ml of the sterile water; and 12.5 ml of the melted 4% Agarose Gel in the 37°C container to make the plaquing overlay. Maintain at 37°C to 42°C. Note: This example provides for a final agarose gel concentration of 1%. The final concentration may be varied from 2% to 0.2% by adjusting the water complement of the overlay solution.

Continue with protocol:

10. Sequentially remove inoculum from each plate with a Pasteur pipette and add 4 ml of the desired plaquing overlay. Note: This must be done quickly to avoid drying of the monolayer and premature gelling of the overlay solution.
11. Allow overlays to solidify (10 to 30 min) at room temperature.
12. Carefully place the plates in a sealed container with a damp cloth (to provide humidity) and incubate at 28°C ± 0.5°C for 4 to 6 days. Note: Wild-type virus produces highly refractile, near-white plaques to the naked eye. Recombinant virus produces milky-gray plaques of slight contrast without staining or other detection methods. Bluo-gal produces a deep-blue precipitate in the immediate area of the β-galactosidase product.